

AMENDMENTS TO THE SPECIFICATION

The amendments below are described relative to the specification filed and including amendments filed on August 17, 1998, December 23, 1999 and December 2, 2002. For clarity, a version of this specification is submitted herewith and labeled as "SPECIFICATION AS FILED WITH AMENDMENTS FILED ON AUGUST 17, 1998, DECEMBER 23, 1999 AND DECEMBER 2, 2002 INCLUDED IN BOLD."

Please amend the specification as shown, without prejudice or disclaimer.

Please insert the following paragraph on page 1 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" immediately before the "Background" section heading:

--INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON COMPACT DISCS

The sequence listing in the present application is being submitted on two compact discs labeled "Sequence Listing-Copy 1" and "Sequence Listing-Copy 2"; each containing a file of 16 KB in size named "226-104 US SEQ LIST" created on September 29, 2005, the contents of which are hereby incorporated by reference.--

Please replace the paragraph beginning at page 5, line 9 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraph:

Non-metabolic actions of amylin include vasodilator effects which may be mediated by interaction with CGRP vascular receptors. Reported in vivo tests suggest that amylin is at least about 100 to 1000 times less potent than CGRP as a vasodilator (Brain et al., *Eur. J. Pharmacol.*, 183:2221 (1990); Wang et al., *FEBS Letts.*, 291:195-198 (1991)). The effect of amylin on regional hemodynamic actions, including renal blood flow, in conscious rats has been reported

(Gardiner et al., *Diabetes*, 40:948-951 (1991)). The authors noted that infusion of rat amylin was associated with greater renal vasodilation and less mesenteric vasoconstriction than is seen with infusion of human α -CGRP. They concluded that, by promoting renal hyperemia to a greater extent than did α -CGRP, rat amylin could cause less marked stimulation of the renin-angiotensin system, and thus, less secondary angiotensin II-mediated vasoconstriction. It was also noted, however, that during ~~[[coinfusion]]~~ coinfusion of human α -⁸⁻³⁷CGRP [SEQIDNO:16] and rat amylin, renal and mesenteric vasoconstrictions were unmasked, presumably due to unopposed vasoconstrictor effects of angiotensin II, and that this finding is similar to that seen during coinfusion of human ~~[[A]]~~ α -CGRP and human α -⁸⁻³⁷CGRP [SEQIDNO:16] (id. at 951).

Please replace the paragraph beginning at page 10, line 7 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraph.

Further, amylin agonist analogues useful in the methods of this application include
amylin agonist analogues having the following amino acid sequence [SEQ ID NO:23]:

Please replace the paragraph beginning at page 13, line 16 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraph:

In a preferred embodiment, the amylin agonist is an amylin agonist analogue, preferably,
^{25, 28, 29}Pro-h-amylin [SEQ ID NO:1].

Please replace the paragraph beginning at page 14, line 2 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraph:

1. An agonist analogue of amylin having the amino acid sequence [SEQ ID NO:17]:

Please replace the paragraph beginning at page 14, line 26 of the “Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold” with the following amended paragraph:

2. An agonist analogue of amylin having the amino acid sequence [SEQ ID NO:18]:

Please replace the paragraph beginning at page 15, line 27 of the “Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold” with the following amended paragraph:

3. An agonist analogue of amylin having the amino acid sequence [SEQ ID NO:19]:

Please replace the paragraph beginning at page 16, line 23 of the “Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold” with the following amended paragraph:

4. An agonist analogue of amylin having the amino acid sequence [SEQ ID NO:20]:

Please replace the paragraph beginning at page 17, line 18 of the “Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold” with the following amended paragraph:

Preferred amylin agonist analogues include, ^{25, 28, 29}Pro-h-amylin [SEQ ID NO:1],

¹⁸Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:2], ¹⁸Arg^{25,28}Pro-h-amylin [SEQ ID NO:3].

Please replace the paragraphs beginning at page 26, line 2 of the “Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold” with the following amended paragraphs:

Preparation of ^{25,28,29}Pro-h-Amylin [SEQ ID NO:1]

Solid phase synthesis of ^{25,28,29}Pro-h-amylin [SEQ ID NO:1] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained

by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25,28,29}Pro-h-amylin [SEQ ID NO:1] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,949.

Please replace the paragraphs beginning at page 26, line 14 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraphs:

Preparation of ¹⁸Arg^{25,28,29}Pro-h-Amylin [SEQ ID NO:2]

Solid phase synthesis of ¹⁸Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:2] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ¹⁸Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:2] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,971.

Please replace the paragraphs beginning at page 26, line 26 of "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraphs:

Preparation of $^{18}\text{Arg}^{25,28}\text{Pro-h-Amylin}$ [SEQ ID NO:3]

Solid phase synthesis of $^{18}\text{Arg}^{25,28}\text{Pro-h-amylin}$ [SEQ ID NO:3] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7 -[disulfide]amylin-MBHA-resin was obtained by treatment of Ac_m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The $^{18}\text{Arg}^{25,28}\text{Pro-h-amylin}$ [SEQ ID NO:3] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,959.

Please replace the paragraph beginning at page 27, line 12 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraph:

Evaluation of the binding of compounds to amylin receptors was carried out as follows. ^{125}I -rat amylin [SEQ ID NO:21] (Bolton-Hunter labeled at the N-terminal lysine) was purchased from Amersham Corporation (Arlington Heights, IL). Specific activities at time of use ranged from 1950 to 2000 Ci/mmol. Unlabeled peptides were obtained from BACHEM Inc. (Torrance, CA) and Peninsula Laboratories (Belmont, CA).

Please replace the paragraph beginning at page 27, line 26 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraph:

To measure ^{125}I -amylin [SEQ ID NO:22] binding, membranes from 4 mg original wet weight of tissue were incubated with ^{125}I -amylin [SEQ ID NO:22] at 12-16 pM in 20 mM

HEPES buffer containing 0.5 mg/ml bacitracin, 0.5 mg/ml bovine serum albumin, and 0.2 mM PMSF. Solutions were incubated for 60 minutes at 23°C. Incubations were terminated by filtration through GF/B glass fiber filters (Whatman Inc., Clifton, NJ) which had been presoaked for 4 hours in 0.3% polyethyleneimine in order to reduce nonspecific binding of radiolabeled peptides. Filters were washed immediately before filtration with 5 ml cold PBS, and immediately after filtration with 15 ml cold PBS. Filters were removed and radioactivity assessed in a gamma-counter at a counting efficiency of 77%. Competition curves were generated by measuring binding in the presence of 10^{-12} to 10^{-6} M unlabeled test compound and were analyzed by nonlinear regression using a 4-parameter logistic equation (INPLOT program; GRAPHPAD Software, San Diego).

Please replace Table II beginning at page 30, line 12 of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended Table II:

TABLE II

	Receptor Binding <u>Assay IC₅₀ (pM)</u>	Soleus Muscle <u>Assay EC₅₀ (nM)</u>
1) ²⁸ Pro-h-Amylin [SEQ ID NO:4]	15.0	2.64
2) ²⁵ Pro ²⁶ Val ^{28,29} Pro-h-Amylin [SEQ ID NO:5]	18.0	4.68
3) ^{2,7} Cyclo-[² Asp, ⁷ Lys]-h-Amylin [SEQ ID NO:6]	310.0	6.62
4) ²⁻³⁷ h-Amylin [SEQ ID NO:7]	236.0	1.63
5) ¹ Ala-h-Amylin [SEQ ID NO:8]	148.0	12.78

6)	¹ Ser-h-Amylin [SEQ ID NO:9]	33.0	8.70
7)	²⁹ Pro-h-Amylin [SEQ ID NO:10]	64.0	3.75
8)	^{25,28} Pro-h-Amylin [SEQ ID NO:11]	26.0	13.20
9)	des- ¹ Lys ^{25,28} Pro-h-Amylin [SEQ ID NO:12]	85.0	7.70
10)	¹⁸ Arg ^{25,28} Pro-h-Amylin [SEQ ID NO:3]	32.0	2.83
11)	des- ¹ Lys ¹⁸ Arg ^{25,28} Pro-h-Amylin [SEQ ID NO:13]	82.0	3.77
12)	¹⁸ Arg ^{25,28,29} Pro-h-Amylin [SEQ ID NO:2]	21.0	1.25
13)	des- ¹ Lys ¹⁸ Arg ^{25,28,29} Pro-h-Amylin [SEQ ID NO:14]	21.0	1.86
14)	^{25,28,29} Pro-h-Amylin [SEQ ID NO:1]	10.0	3.71
15)	des- ¹ Lys ^{25,28,29} Pro-h-Amylin [SEQ ID NO:15]	14.0	4.15

Please replace the paragraph beginning at page 32, line 11, of the “Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold” with the following amended paragraph.

In dose response studies, rat amylin (Bachem, Torrance, CA) dissolved in 0.15 M saline, was administered as a 0.1 mL subcutaneous bolus in doses of 0, 0.01, 0.1, 1, 10 or 100 µg 5 minutes before gavage in Harlan Sprague Dawley (non-diabetic) rats fasted 20 hours and diabetic BB rats fasted 6 hours. When subcutaneous amylin injections were given 5 minutes before gavage with phenol red indicator, there was a dose-dependent suppression of gastric

emptying (data not shown). Suppression of gastric emptying was complete in normal HSD rats administered 1 μg of amylin, and in diabetic rats administered 10 μg ($P = 0.22, 0.14$). The ED_{50} for inhibition of gastric emptying in normal rats was 0.43 μg (0.60 nmol/kg) ± 0.19 log units, and was 2.2 $[[\mu]] \mu\text{g}$ (2.3 nmol/kg) ± 0.18 log units in diabetic rats.

Please replace the paragraphs beginning at page 33, line 6, of the "Specification as Filed with Amendments Filed on August 17, 1998, December 23, 1999 and December 2, 2002 Included in Bold" with the following amended paragraphs.

To assist in understanding the present invention, the following further Examples ~~[[A-N]]~~ 9-22 are included and describe the results of a series of experiments therein. The following examples relating to this invention should not, of course, be construed as specifically limiting the invention. Such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the present invention as hereinafter claimed.

EXAMPLE ~~[[A]]~~ 9

Preparation of $^{28}\text{Pro-human-Amylin}$ [SEQ ID NO:4]

Solid phase synthesis of this analogue of human ("h-") amylin using methylbenzhydrylamine anchor-bond resin and N^{α} -Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7 -[disulfide]amylin-MBHA-resin was obtained by treatment of Ac-m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid hydrofluoric acid ("HF") in the presence of dimethylsulfide and anisole. The $^{28}\text{Pro-h-amylin}$ [SEQ ID NO:4] was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed

by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+1)/e=3914.

EXAMPLE [[B]] 10

Preparation of $^{25}\text{Pro}^{26}\text{Val}^{28,29}\text{Pro-h-Amylin}$ [SEQ ID NO:5]

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7 -[disulfide]amylin-MBHA-resin was obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The $^{25}\text{Pro}^{26}\text{Val}^{28,29}\text{Pro-h-amylin}$ [SEQ ID NO:5] was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+1)/e=3936.

EXAMPLE [[C]] 11

Preparation of $^{2,7}\text{Cyclo-}[^2\text{Asp}, ^7\text{Lys}]\text{-h-Amylin}$ [SEQ ID NO:6]

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. ^2Asp and ^7Lys were introduced with Boc- $^2\text{Asp}(\text{Fmoc})\text{-OH}$ and Boc- $^7\text{Lys}(\text{Fmoc})\text{-OH}$. Following selective side-chain deprotection with piperidine, the side-chain to side-chain ($^2\text{Asp-}^7\text{Lys}$) cyclization was carried out using benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent). Cyclization was as described in Di Maio, J., *et al.*, J. Med. Chem., **33**:661-667 (1990); and Felix, A.M., *et al.*, Int. J. Pept. Prot. Res., **32**:441

(1988). The 2,7 cyclo-[2 Asp, 7 Lys]amylin-MBHA-resin obtained after cyclization was cleaved with liquid HF in the presence of dimethylsulfide and anisole. The 2,7 cyclo-[2 Asp, 7 Lys]-h-amylin [SEQ ID NO:6] was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. FAB mass spec: (M+1)/e=3925.

EXAMPLE [[D]] 12

Preparation of des- 1 Lys-h-Amylin [SEQ ID NO:7]

Solid phase synthesis of des- 1 Lys-h-amylin (also represented as $^{2-37}$ h-amylin) [SEQ ID NO:7] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7 -[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des- 1 Lys-h-amylin [SEQ ID NO:7] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,775.

EXAMPLE [[E]] 13

Preparation of 1 Ala-h-Amylin [SEQ ID NO:8]

Solid phase synthesis of 1 Ala-h-amylin [SEQ ID NO:8] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7 -[disulfide]amylin-MBHA-resin was obtained by treatment of

Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ¹Ala-h-amylin [SEQ ID NO:8] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,847.

EXAMPLE [[F]] 14

Preparation of ¹Ser-h-Amylin [SEQ ID NO:9]

Solid phase synthesis of ¹Ser-h-amylin [SEQ ID NO:9] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ¹Ser-h-amylin [SEQ ID NO:9] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,863.

EXAMPLE [[G]] 15

Preparation of ²⁹Pro-h-Amylin [SEQ ID NO:10]

Solid phase synthesis of this analogue of human amylin using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-

protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ²⁹Pro-h-amylin [SEQ ID NO:10] was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3916.

EXAMPLE [[H]] 16

Preparation of ^{25,28}Pro-h-Amylin [SEQ ID NO:11]

Solid phase synthesis of ^{25,28}Pro-h-amylin [SEQ ID NO:11] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Ac^m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25,28}Pro-h-amylin [SEQ ID NO:11] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,939.

EXAMPLE [[H]] 17

Preparation of des-¹Lys^{25,28}Pro-h-Amylin [SEQ ID NO:12]

Solid phase synthesis of des-¹Lys^{25,28}Pro-h-amylin [SEQ ID NO:12] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried

out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys^{25,28}Pro-h-amylin [SEQ ID NO:12] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,811.

EXAMPLE [J] 18

Preparation of des-¹Lys¹⁸Arg^{25,28}Pro-h-Amylin [SEQ ID NO:13]

Solid phase synthesis of des-¹Lys¹⁸Arg^{25,28}Pro-h-amylin [SEQ ID NO:13] using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys¹⁸Arg^{25,28}Pro-h-amylin [SEQ ID NO:13] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,832.

EXAMPLE [[K]] 19

Preparation of des-¹Lys¹⁸Arg^{25,28,29}Pro-h-Amylin [SEQ ID NO:14]

Solid phase synthesis of des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:14] using methylbenzhydramine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Ac^m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:14] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,843.

EXAMPLE [[L]] 20

Preparation of des-¹Lys^{25,28,29}Pro-h-Amylin [SEQ ID NO:15]

Solid phase synthesis of des-¹Lys^{25,28,29}Pro-h-amylin [SEQ ID NO:15] using methylbenzhydramine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Ac^m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys^{25,28,29}Pro-h-amylin [SEQ ID NO:15] was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was

confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^+ = 3,823$.

EXAMPLE [[M]] 21

Preparation of des-¹Lys²⁵Pro²⁶Val^{28,29}Pro-h-Amylin [SEQ ID NO:25]

Solid phase synthesis of this h-amylin analogue using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection is carried out by standard peptide synthesis methods, and the ^{2,7}-[disulfide]amylin-MBHA-resin obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization is achieved, the resin and side chain protecting groups are cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys²⁵Pro²⁶Val^{28,29}Pro-h-amylin [SEQ ID NO:25] is then purified by preparative HPLC.

EXAMPLE [[N]] 22

Preparation of [(D)-¹¹Arg]-Amylin [SEQ ID NO:24]

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection is carried out by standard peptide synthesis methods. (D)-¹¹Arg is introduced with Boc-(D)-¹¹Arg(Mtr)-OH. The ^{2,7}-[disulfide]amylin-MBHA-resin, obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid, is cyclized and the resin and side chain protecting groups are cleaved with liquid HF in the presence of dimethylsulfide and anisole. The [(D)-¹¹Arg]-amylin [SEQ ID NO:24] is then purified by preparative HPLC.